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Mitogen-activated protein kinase 4 of *Leishmania* parasite as a therapeutic target

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ABSTRACT

Protein kinases are important regulators of many different cellular processes such as transcriptional control, cell cycle progression and differentiation, and have drawn much attention as potential drug targets. *Leishmania mexicana* mitogen-activated protein kinase 4 (LmxMPK4) is crucial for the survival of the parasite. As the crystal structure of the enzyme is not known, we have used bioinformatics techniques to model LmxMPK4 structure. The current study reveals conservation of all sequence and structural motifs of LmxMPK4. Study shows mitogen-activated protein kinases are highly conserved throughout different *Leishmania* species and significant divergence is observed towards mammalian mitogen-activated protein kinases. Additionally, using virtual docking methods, we have identified inhibitors for LmxMPK4. The sequence and structure analysis results were helpful in identifying the ligand binding sites and molecular function of the *Leishmania* specific mitogen-activated protein kinase.

1. Introduction

Leishmania is a protozoan parasite of genera Kinetoplastida and exists in two forms, promastigote which infects mammalian host and the amastigote which grows and multiply in host macrophage. The parasite causes several form of leishmaniasis, ranging from self healing cutaneous leishamniasis to potentially lethal visceral form [1–4]. Current therapeutics against the disease is limited, costly and their use is restricted by prevalence of drug resistant strains. In the search for novel therapeutics against the disease the requirement is not only to rationally design new therapeutic agents with existing targets but also identify and validate potentially new drug targets for novel drug design [5–7]. Protein kinases have been long studied for their potential role in growth, differentiation, survival, apoptosis and cellular stress responses thus they are key regulatory molecules in signal transduction cascade of eukaryotic cells. Mitogen-activated protein kinases (MAPKs) are a class of serine and/or threonine protein kinases involved in transduction of signals from environmental stimuli by phosphorylation and dephosphorylation of specific kinases, in turn they regulate the protein expression profile of the organism [8–13]. A MAPK homologue of *Leishmania mexicana*, LmxMAPK4, is reported to be essential for proliferation and survival of promastigote and amastigote forms of the parasite [14–17]. Genome sequencing and analysis of *Leishmania major* and *Leishmania infantum* revealed the presence of all members of the MAPK signal transduction cascade [18] and it is not yet known how regulation of gene expression is achieved in parasites. *L.mexicana* MAPKs possess carboxy-terminal extensions comprising 52 to 1186 amino acids. Long carboxy-terminal extensions have also been found in mammalian MAPKs ERK5 (400 amino acids), ERK7 (195 amino acids) and ERK8 (194 amino acids) [19–22]. The extention is likely to play a regulatory role in ERK5 as only a truncated version shows in vitro activity [23]. In ERK7 the carboxy-terminal domain is required for the cellular localization and functions as a negative regulator of growth [21]. Nothing is known yet about the function of these extensions in *Leishmania*. MAPKs are focus of novel drug discoveries. Recently, modelled structure and potential inhibitors of CRK3 cyclin-dependent kinase of *Leishmania*, distant member of the MAPK superfamily was published [24].

As structure (X-ray or NMR) of LmxMPK4 is not available, we have performed homology modelling of LmxMPK4 based on the X-ray crystallographic structure of the human ERK2. The study presents the 3D structure of LmxMPK4 and reveals sequence and structural conservation of MAPK in *L.mexicana*. This study also revealed the structural differences between human ERK and

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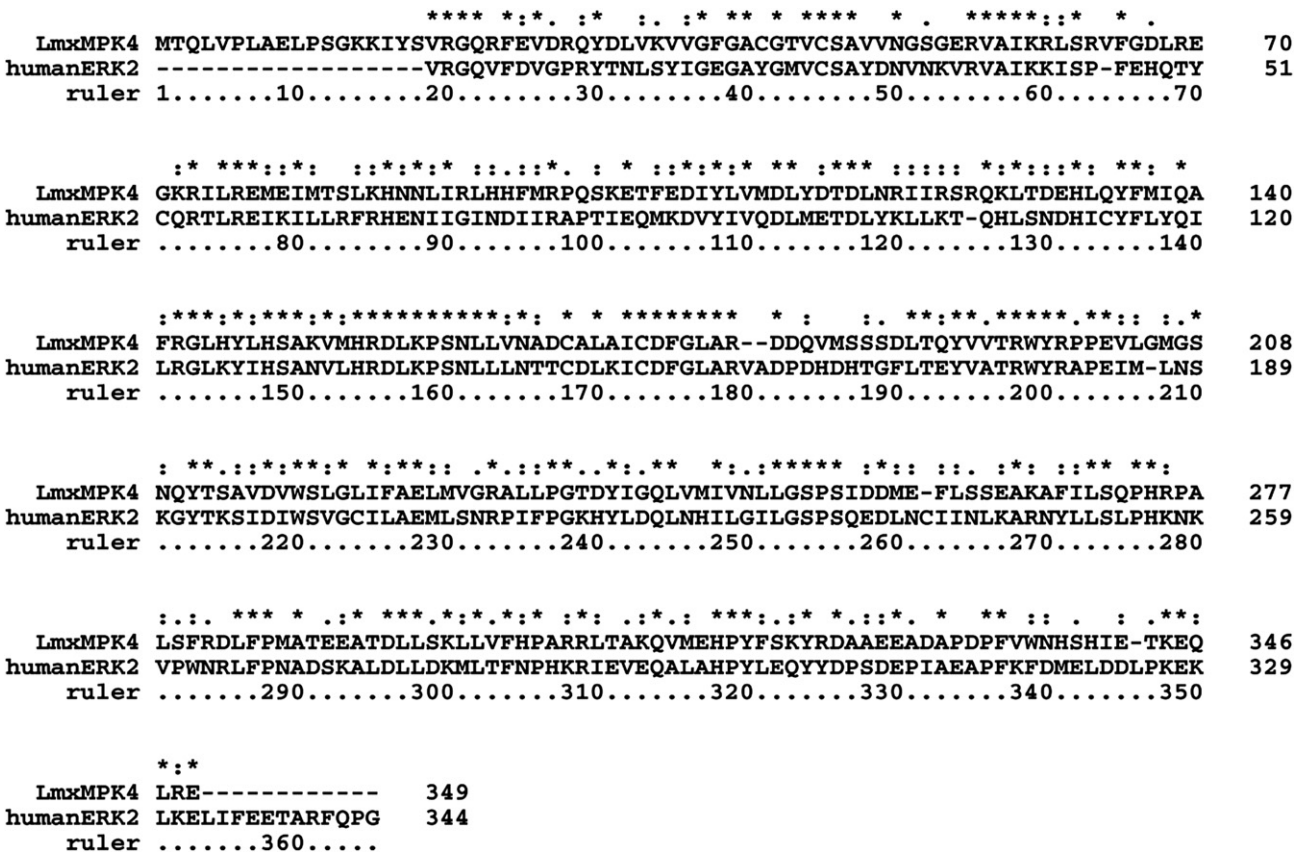

Fig. 1. Sequence alignment of LmxMPK4 and its template human ERK2. Stars indicate identical amino acids while colon for similar amino acids and single dot for nearly similar ones. The sequence alignment of LmxMPK4 and human ERK2 (template) shows that the protein kinase domain is conserved. N-terminal flanking sequence of LmxMPK4 (18 amino acids) does not align with human ERK2 (No structural information in PDB with ID 1TVO).

Leishmania LmxMPK4. Further, we report identification of several novel compounds that can inhibit LmxMPK4 with an acceptable inhibition range. Identified compounds may be useful analytical tools, to elucidate the roles of LmxMPK4 in intracellular signaling pathways, as well as leads for pharmaceuticals. Investigation of its structure proved this kinase as a promising drug target to treat leishmaniasis by preventing the proliferation of the parasites and hence cures the disease.

2. Materials and methods

2.1. Sequence analysis

LmxMPK4 (Accession Number: Q9GRU1) along with 16 other paralog sequences from *L.mexicana* were retrieved from UniProtKB database (http://www.uniprot.org/). To identify conserved motifs, 17 *Leishmania* MAPKs were analyzed with the MEME program (http://meme.sdsc.edu/) [25], which uses expectation-maximization to discover motifs in sets of unaligned protein sequences. Multiple sequence alignment was generated using Muscle (http://www.drive5.com/muscle/) [26].

2.2. Homology modelling

As Kinetoplastid MAPKs structures are not known, human ERK2 (PDB code: 1TVO) served as template for the homology modelling process based on its sequence similarity to LmxMPK4, the template was identified using NCBI-BLASTP search against Protein Data Bank (PDB) with default parameters. Selection of human ERK2 an extracellular signal regulated kinase as template for structural modelling was due to its homology (42% and 65% identity and similarity, respectively). The crystal structure of human ERK2 was solved at structural resolution of 2.50 Å and is in complex with the 5-(2-phenylpyrazolo [1,5-a] pyridin-3-yl)-1h-pyrazolo [3,4-c] pyridazin-3-amine ligand (Ligand Id. FRZ) [22]. The sequence alignment of LmxMPK4 and human ERK2 (template) shows that the protein kinase domain is conserved in both LmxMPK4 and human ERK2 which extends from residues Val36 to Tyr317 in LmxMPK4 (Fig. 1). There is a difference of eighteen amino acids in the sequence and structural information of LmxMPK4 due to non-availability of structural information in human ERK2 to model LmxMPK4 N-terminal eighteen amino acids (Val19 is considered to be Val1 in LmxMPK4 structure). Sequence alignment obtained from NCBI-BLASTP was used as an input for initial automated model building in MODELLER9v6 (http://salilab.org/modeller/) based on satisfaction of spatial restraints [27]. Quality of the hundred 3D structure homology models with its ligand were ranked by Discrete Optimized Protein Energy (DOPE) score and best models which has low DOPE score was assessed using PROCHECK validation package (http://nihserver.mbi.ucla.edu/SAVES/), in order to identify the best ligand supported LmxMPK4 model [28]. This model showed problem in the loop region which was subjected for loop modelling. After each round of loop modelling, 100 models were generated which were then further validated for low DOPE score. This iteration process continued until most of the amino acid residues have a cut-off value below 95% in ERRAT plot [29]. Final LmxMPK4 model showing best PROCHECK and ERRAT plot was then subjected to native protein folding energy evaluation using ProsaII program [30].